

UNIVERSITI TEKNOLOGI MARA

**ENHANCED PERFORMANCE OF
ENCAPSULATED MULTI-ENZYME
IN INDIGENOUS SAYONG CLAY
FOR CASSAVA
SACCHARIFICATION PROCESS**

SITI NORAI DA BINTI ABD RAHIM

Thesis submitted in fulfillment
of the requirements for the degree of
Master of Science

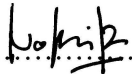
Faculty of Plantation and Agrotechnology

June 2015

AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

Name of Student	:	Siti Noraida Binti Abd Rahim
Student I.D. No.	:	2011642264
Programme	:	Master of Science (By Research)
Faculty	:	Plantation and Agrotechnology
Thesis Title	:	Enhanced Performance of Encapsulated Multi-enzyme in Indigenous Sayong Clay for Cassava Saccharification Process
Signature of Student	:	
Date	:	June 2015

ABSTRACT

The encapsulation of multi-enzyme (alpha-amylase, glucoamylase and cellulase) within calcium alginate-clay beads was used in the saccharification of cassava into reducing sugar in a stirred tank bioreactor. The functional group, morphology and composition of beads were characterized by Fourier Transform Infrared Spectroscopy (FTIR) and Field Emission Scanning Electron Microscope (FESEM) equipped with Energy Dispersive X-ray Spectroscopy (EDX). The optimal encapsulation conditions of multi-enzyme in alginate-clay beads were obtained at 2 % (w/v) clay concentration and 0.2 M CaCl_2 solution which gave 97.34 % of loading efficiency and 53.71 % of immobilization yield. Kinetic parameters were determined for encapsulated multi-enzyme, as well as for the free multi-enzyme. The Michaelis constant, K_m value for encapsulated multi-enzyme (4.9176 mg/mL) was 1.39 times higher than free multi-enzyme (3.5367 mg/mL), whereas the maximum reaction velocity, V_{\max} value was lower for the encapsulated multi-enzyme. Based on the one-factor-at-one-time (OFAT) study, the optimum pH, temperature and agitation speed for both free and encapsulated multi-enzyme were pH 6.0, 50 °C and 120 rpm, respectively. Furthermore, the optimum conditions for saccharification process were statistical determined using Response Surface Methodology (RSM). After screening significant factors that influence the saccharification process, pH, temperature and agitation speed were optimized by implementing central composite design (CCD) in RSM. The optimum values of pH, temperature and agitation speed were found to be pH 5.9, 44 °C and 94 rpm, respectively. Under these conditions, the experimental yield was 88.19 % which was close to the predicted yield (91.95 %). Meanwhile, encapsulated multi-enzyme was retained 15.09 % of its activity after 5 reaction cycles.

TABLE OF CONTENTS

	Page
CONFIRMATION BY PANEL OF EXAMINERS	ii
AUTHOR'S DECLARATION	iii
ABSTRACT	iv
ACKNOWLEDGEMENT	v
TABLE OF CONTENTS	vi
LIST OF TABLES	x
LIST OF FIGURES	xi
LIST OF PLATES	xiv
LIST OF SYMBOLS	xv
LIST OF ABBREVIATIONS	xvii
CHAPTER ONE: INTRODUCTION	
1.1 Research Background	1
1.2 Problem Statement	4
1.3 Objectives of the Research	5
1.4 Scope and Limitation of the Research	5
1.5 Thesis Overview	6
CHAPTER TWO: LITERATURE REVIEW	
2.1 Cassava Root	7
2.1.1 Composition of Cassava Root	8
2.1.2 World Production of Cassava Root	10
2.1.3 Application of Cassava Root	10
2.2 Enzyme	11
2.2.1 Alpha-amylase	13
2.2.2 Glucoamylase	14
2.2.3 Cellulase	15
2.2.4 Multi-enzyme	17

2.3	Enzyme Immobilization	18
2.3.1	Immobilization Techniques	19
2.4	Supporting Material	21
2.4.1	Alginate	22
2.4.2	Kaolinite Clay	23
2.5	Enzyme Bioreactor	24
2.5.1	Types of Enzyme Bioreactor	25
2.5.2	Application of Enzyme Bioreactor	27
2.6	Hydrolysis Process	29
2.6.1	Factors Affecting Enzymatic Reaction	30
2.7	Optimization Method	33
2.7.1	One-factor-at-one-time	33
2.7.2	Response Surface Methodology	33
 CHAPTER THREE: MATERIALS AND METHODS		
3.1	Materials	36
3.2	Research Methodology	37
3.3	Preparation of Cassava Slurry	38
3.4	Characterization of Cassava Root	38
3.4.1	Moisture Content	38
3.4.2	Starch Content	38
3.4.3	Amylose and Amylopectin Content	39
3.4.4	Crude Fibre Content	39
3.4.5	Reducing Sugar Content	40
3.4.6	Protein Content	41
3.5	Determination of Enzyme Activity	41
3.5.1	Preparation of Enzyme Solution	41
3.5.2	Alpha-amylase	42
3.5.3	Glucoamylase	42
3.5.4	Cellulase	42
3.6	Preparation of Calcium Alginate-clay Beads	43
3.7	Characterization of Calcium Alginate-clay Beads	43
3.7.1	FTIR Analysis	43